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Effect of nefopam in normal chickens and its relationship to hydrogen peroxide-induced oxidative stress

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Abstract

The objective designated to discover the analgesic effect of nefopam in the normal (non-stressed) chickens and its possible alteration due to hydrogen peroxide (H_2O_2)-induced oxidative stress (OS) in 7-14 day old chickens. The analgesia of nefopam has been increased by 47% in the stressed chickens by measuring the analgesic Median Effective Dose (ED_{50}) value. This value was 9.10 mg/kg, IM in the normal chickens where it became 4.80 mg/kg, IM in stressed chickens. There is a significant rise in the antinociceptive action of nefopam 18 mg/kg, IM by 88% in the stressed group of chickens in comparison with the normal one elicited by an electro-stimulation and formaldehyde 0.05 ml of 0.1% tests for induction of nociception. The observations showed several significant stimulatory modifications in the neurobehaviour when nefopam treated with a subtle dosage 1 mg/kg, IM in the stressed chickens concerning the latency to move, squares crossed and time of the tonic immobility response test. Significant damage was detected in the liver function when nefopam injected at 18 mg/kg, IM in stress chickens in comparison to normal one by 28, 33 and 65% as estimated through Alkaline phosphatase (ALP), Aspartate trans-aminase (AST) and Alanine trans-aminase (ALT) concentrations in the serum, respectively. The sum of data findings indicated that H_2O_2-induced OS increased the analgesic activity of nefopam in the chickens; despite the changes occur on the neurobehaviour and liver function. The dose of nefopam should be reduced when preparing the therapeutic regimen in the stressed animals.

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Introduction

Nefopam is considered a non-narcotic analgesic medication used primarily to treat moderate to severe of acute or chronic nociception and to treat of a neuropathic pain disorder (1). Unlike narcotics; it does not causes addiction, respiratory depression and other deleterious effects related to narcotics therapy (1) and its analgesic activity may be potentiated by acetaminophen coadministration (2). By its centrally acting on the brain and spinal cord, nefopam could produce a better, more profound and reliable analgesia without causing respiratory depression similarly to morphine (1,3-5). Nefopam works by a unique mechanism of action by which it produces analgesia through either modification of Na^{+2} and Ca^{+2} canals which decrease glutamate release that considered an important neuro-transmitter associated with noci-ceptive processing or it elevates catecholamines (especially norepinephrine and dopamine) and serotonin activity by inhibiting their reuptake from the presynaptic neurons, which are well-thought-out a pain signaling dependent neurotransmitters (1,5). Many stressful conditions such as chemical (H_2O_2) and physical (heat) stressors are known to cause modulations in drug response, especially for the centrally acting drugs (11). H_2O_2 was previously known to modulate the sedative action of diazepam (6) and xylazine

(7) and altered the anesthetic properties of ketamine anesthesia in the chickens, which have some deleterious side effects toward the animals (8) in addition to modifying xylazine and diazepam efficacy (9), especially for the drugs with narrow the margin of safety. H_2O_2 are well organized to cause OS by increasing reactive O_2 species (ROS), thus increases the content of free radicals which interacts and modifies the functions of the cellular components especially the receptor proteins responsible for pharmacodynamics and the cytochrome P_{450} enzymes responsible for pharmacokinetics (10,11) of the drugs and destruction of the blood-brain barrier (12) and thus altering the drug response.

Because nefopam having no addiction and not causes respiratory depression with efficient analgesia, the purpose of this study consisted of using nefopam as a first report in normal (non-stressed) chickens to determine the beneficial effect of nefopam in the veterinary medicine; despite the possible alteration in the analgesic drug response in case of H_2O_2-induced OS in the stressed chickens.

Materials and methods

Experimental chickens and chemicals

Both genders of broiler chickens at 7-14-day-old were used in all the trials which supplied from a local hatchery with a regular body weight between 72–110 g. They were preserved at 30-36ºC, ceaseless light. Experimental chickens have permitted ad libitum to water and ration. Nefopam (1%, Nefopam chlorhydrate, France) extenuating with a normal saline (0.9% NaCl) to be injected intramuscularly (IM) as volume 5 ml/kg.

Ethics

A methodology for the research and the use of experimental chickens has been authentic by professional committee of the Veterinary Medicine College at the University of Mosul on ethical quality for medical research considerations.

OS status and its induction in the chickens with H_2O_2

One day old chickens were randomly separated into a normal group (H_2O) which provided a water whereas the other stress chickens (H_2O_2) had provided daily fresh 0.5% H_2O_2 (Scharlab, Spain) in drinking water. Prior literatures revealed, incessant 0.5 % H_2O_2/day in water, can induce OS once assumed for chickens from 1st to 14th-day-old. This treatment pattern of H_2O_2 induces OS at day 7^{th}, 10^{th} and 14^{th} of chickens confirmed all through by OS biomarkers which were the decrease in glutathione level and the increase of concentrations of malondialdehyde of brain and plasma samples of the chickens by way of OS pointer (6,8). For the above-mentioned reasons; 7-14-days old chickens casted off in the subsequent trials.

Nefopam's ED50 for analgesia of normal and stressed chickens

The analgesic ED_{50} value of nefopam was carried out as the first step by ascending and descending way (13) to normal and stressed chicken's groups for choosing the nefopam dosage so that, this dosage will used in the following trials. The initial nefopam dosage at 10 mg/kg, IM (2,3) for both groups of chickens by rise or reduce in the dosage as 3 mg/kg (not more than 30% of the initial dose). Nefopam analgesia was determined by using an electro-stimulator device (Harvard Apparatus, USA) as pain-inducing in the chickens (6,8,11). Before and after 30 minutes of nefopam injection, the distress call produced because of noci-ception noted by way of a voltage for every chicken individually. Nefopam considered possesses an anti-nociceptive effect as well as the voltage augmented post-treatment compared to volts recorded pre-treatment which marked as X and if it is not, it will be designated as O. Subsequent equation casted off to quantify influence of the OS for nefopam's analgesic ED_{50}: % OS effect to nefopam's ED_{50}= ED_{50} of H_2O group- ED_{50} of H_2O_2 group/ ED_{50} of H_2O group × 100.

The antinociception of nefopam against electro-stimulation in normal and stressed chickens

One dose of nefopam was chosen at 18 mg/kg, IM was injected to 6 chickens per H_2O or H_2O_2 groups depending on the previous experiment similar to the analgesic ED_{100} values of nefopam. The voltage of electro-stimulator apparatus registered pre- and post-thirty min of nefopam therapy to every chicken although the delta volts also noted (6,7,14-19). Over the previously mentioned calculation, the percentage contributed to H_2O_2-induced OS for nefopam's analgesia can be measured by using the delta voltage for H_2O and H_2O_2 groups of chickens.

Antinociceptive effect of nefopam against formaldehyde induced pain sensation in the right paw of normal and stressed chickens

Nefopam (18 mg/kg, IM) was treated to 6 chickens/group. After 15 minutes of nefopam injection, formaldehyde (0.1%) was injected at 0.05 ml in the right paw of both the normal and stressed groups. After 15 minutes of formaldehyde injection to the right paw, the latency of right paw lifting (seconds), a number of right paw lifting and the longer period from lifting to descending of the right paw (duration) (seconds) were documented for 3 minutes for each chick individually as previously described (20).

The neurobehavioral effects of nefopam in normal and stressed chickens

Different measurements of the open-field activity were used to investigate the acute pattern of the neurobehavioral changes of nefopam in normal and stressed groups (21).

Activity in open field of all chickens observed 30 minutes afterward nefopam therapy (1 mg/kg, IM) and this dose did not produce an obvious effect, thus, the subtle effect can be determined to resemble the neurobehavioral deficits (21). Each chicken was positioned alone centrally in field box (60×60×30cm with box floor divided to 16 squares), spreading 50 grams of feed grains on it. Measurements included the subsequent neurobehavioral parameters which observed afterward 30 min of nefopam injection within five minutes interval which included the moving starting in seconds, amount of squares traversed by two legs, an escape jumping, a number of defecations, distress calls and pecking. Afterward, each chicken exposed to examination of tonic immobility (21) by catching every chicken by two hands, setting the chicken at woody surface.

Measurement the ALP, ALT and AST concentrations in normal and stressed chickens treated with nefopam

Four hours after nefopam therapy at 18 mg/kg, IM, the samples of blood attained of the vein of normal besides stressed groups of chickens (6 chickens/group) and driven in the gel tubes to be centrifuged (Chalice, UK). The gotten samples of serum exploit to estimate serum ALP, AST and ALT (22) levels in Unit/Liter (U/L) which determined using Chemistry analyzer Smart-150 apparatus (Genotek, USA) to determine the possible deleterious effects of nefopam in normal and stressed chickens.

Statistics

Paired and unpaired student T-test implemented to relate the means of two groups of parametric data whereas the non-parametric data were statistically analyzed by the mann-whitney-U-test (23). Significance accounted of all data stand for $P < 5\%$.

Results

Analgesic ED_{50} of nefopam

Nefopam analgesic efficacy has been modified and increased in the stressed (H_2O_2) group of chickens concluded by measuring the analgesic ED_{50} value of nefopam. The value was at 9.10 mg/kg, IM in the normal (H_2O) group while it declined by 47% in the stressed group of chickens because of the effect of OS induced with H_2O_2 to be 4.80 mg/kg, IM as showed in Table 1.

Analgesic effect of nefopam in normal and stressed chickens

Table 2 shows the antinociceptive action of nefopam increased in its efficacy by 88% in stressed chickens after 30 minutes of nefopam injection at 18 mg/kg for the effect of H_2O_2-induced OS. There is a significantly difference of elevation of post-injection nefopam's anti-nociception and Δ voltage provoked through electro-stimulation associated to normal group.

Table 1: Analgesic ED_{50} value of nefopam in normal and stressed chickens

Parameter	Result	
	Normal group	stressed group
ED_{50} value= xf + K × d	9.10 mg/kg, IM	4.80 mg/kg, IM
Doses extent	7-13 mg/kg	4-10 mg/kg
Early dosage	10 mg/kg	10 mg/kg
Latter dosage (xf)	9 mg/kg	7 mg/kg
K table from (20)	-0.305	-0.737
± in the dose (d)	3 mg	3 mg
Chickens quantity	5 (XOOXX)*	6 (XXOXOX)*
OS's effect of nefopam's anti-nociceptive ED_{50}= normal-stressed/normal×100= 47%		

Plain tab water was given to the normal (H_2O) group while 0.5% H_2O_2 in water was supplemented the stressed (H_2O_2) group from 1st to 14th-day-old in chickens' age. *X= analgesia, O= no analgesia.

Table 2: Nefopam's analgesia in normal and stress chickens models

Variable	Anti-nociception%	Pre-therapy volts	Post-therapy volts	Δ Voltage
Normal (H_2O) group	100 (6/6)	8.17 ± 0.54	12.33 ± 0.71[a]	4.17 ± 0.31
Stressed (H_2O_2) group	100 (6/6)	9.17 ± 0.48	17.73 ± 0.73 *,[a]	7.83 ± 0.40*
OS's effect for nefopam's analgesia = Δ stressed-Δ normal/Δ normal × 100= 88%				

Numbers denoted mean ± Std.Err. 0.5% H_2O_2 in water was supplemented the stressed (H_2O_2) group from 1st to 14th-day-old in chickens' age. Post-injection record of volts was after 30 minutes of nefopam was injected at 18 mg/kg, IM for both the normal and stressed groups. * Differ significantly as of the normal chickens (P<5%). A differ significantly as of pre-therapy at the similar group of chickens (P < 5 %).

Nefopam's antinociception against formaldehyde induced pain sensation in the right paw of normal and stressed chickens

A significant augmentation in the nefopam antinociceptive efficacy was noticed in the stressed chickens compared to the normal chickens as presented by recording the latency of right paw lifting, it's lifting number and the duration of the right paw lifting that injected with the irritant formaldehyde (Table 3).

Neurobehavioral effects of nefopam in normal and stressed chickens

Administration of nefopam by subtle (1 mg/kg, IM) dosage causes different significant neurobehavioral changes in the stressed chickens concerning the beginning of moving in seconds.

An amount of squares intersected with two legs and the time of the tonic immobility response test in seconds while there is a slight difference on a number of defecation, numbers of escape jumps and pecking behaviour which refer to rise in the subtle stimulant effect of nefopam in stressed chickens compared to the normal chickens as noticed in Table 4.

Serum ALP, ALT and AST concentrations for determination of liver function in normal and stressed chickens treated with nefopam

Table 5 demonstrated there is significantly differs of elevation of ALP, ALT and AST concentrations of serum the stressed chickens by 28, 65 and 33% when compared to the normal group of chickens that both treated with nefopam at 18 mg/kg, IM which shows the serious deleterious effect on the liver function due to the effect of H2O2-induced OS criteria in the stressed chickens.

Table 3: Antinociceptive effect of nefopam against formaldehyde induced pain sensation in the right paw of normal and stressed chickens

Groups	Latency of the right paw lifting (seconds)	Number of the right paw lifting	Duration of the right paw lifting (seconds)
Normal (H_2O) group	19.50 ± 2.20	15.67 ± 1.57	1.47 ± 0.21
Stressed (H_2O_2) group	48.10 ± 4.67*	3.43 ± 0.19*	1.00 ± 0.00*

Numbers denoted mean ± Std.Err. 0.5% H_2O_2 in water was supplemented the stressed (H_2O_2) group from 1st to 14th-day-old in chickens' age. Formaldehyde (0.05 ml of 0.1%) was injected in the right paw after 15 minutes of nefopam injection at 18 mg/kg, IM. The above data recorded after 30 minutes of nefopam injection for 3 minutes. * Differ significantly as of the normal chickens (P<5%).

Table 4: Nefopam's neurobehavioral deficits at normal along with stress chickens

Neurobehavioral Parameters	Groups	
	Normal group	Stressed group
Latency to move (seconds)	11.33 ± 0.49	5.17 ± 1.25*
Number of squares crossed by both legs	29.83 ± 2.85	47.17 ± 3.56*
Tonic immobility response (seconds)	4.50 ± 0.34	2.33 ± 0.21*
Number of defecation	0.33 ± 0.21	0.67 ± 0.21
Number of escape jumps	0.67 ± 0.21	1.00 ± 0.37
Distress-calls	3.00 ± 0.00	3.00 ± 0.00
Pecking scores	0.67 ± 0.21	1.33 ± 0.33

The values denoted Mean ± S.E. 0.5% H_2O_2 in water was supplemented the stressed (H_2O_2) group from 1st to 14th-day-old in chickens' age. Open field activity was recorded after 30 minutes of nefopam injection at 1 mg/kg, IM for both the normal besides stress groups. * Differ significantly as of the normal chickens (P<5%).

Table 5: Serum ALP, ALT and AST concentrations in normal and stressed chickens treated with nefopam

Variable groups	ALP (U/L)	ALT (U/L)	AST (U/L)
Normal (H_2O) group	227.50 ± 1.88	12.33 ± 0.61	216.00 ± 3.79
Stressed (H_2O_2) group	292.00 ± 4.34*	20.33 ± 0.76*	287.67 ± 3.65*
Effect of OS on liver function parameters (%)= stressed-normal/normal×100	28	65	33

The values denoted Mean ± S.E. 0.5% H_2O_2 in water was supplemented the stressed (H_2O_2) group from 1st to 14th-day-old in chickens' age. Nefopam was injected at 18 mg/kg, IM. * Differ significantly as of the normal chickens (P<5%).

Discussion

The purpose of this study consisted from using nefopam as a first report in normal (non-stressed) chickens to determine the beneficial effect of nefopam in the veterinary medicine; despite the possible alteration in the analgesic drug response in case of H_2O_2-induced OS in the stressed chickens because nefopam having no addiction and not causes respiratory depression with efficient analgesia (1). Nefopam primarily used to treat moderate-to-severe acute or chronic nociception (1) and to treat of the neuropathic pain disorders (1,24) and postoperative pain therapy (1) through its central action on the central nervous system (CNS). As noticed, the central antinociceptive effect of nefopam was increased in efficacy in the stressed chickens (0.5% H_2O_2 added to their water) compared to the normal chickens (supplied with tap water) concerning ED_{50} value, electro-stimulation and formaldehyde induced pain sensation. This trial used H_2O_2 as a well-known powerful oxidant for inducing of OS experimentally (6). Nefopam has been selected because of its centrally acting mechanism by which it produces analgesia through either modification of Na^{+2} and Ca^{+2} canals which diminish the glutamate releasing elaborate in nociceptive handling or it elevates norepinephrine, dopamine and serotonin activity by inhibiting their reuptake from the presynaptic neurons are considered in pain signaling (1,7). The CNS-acting drugs were found to be the most altered in pharmacological response due to the OS status that induced by H_2O_2 because of its ability to destruct the Blood-Brain Barrier (15) and thus making the CNS receptors more vulnerable to high drug concentration and later augmented the drug affinity and efficacy as these findings agree with earlier studies in the same field (9-12).

Other reasons implemented to the increase of the nefopam efficacy refers to H_2O_2 ability to cause OS by elevating ROS; thus increases the free radicals formation which interacts and alters the functions of the cellular components especially the receptor proteins that involved in drug pharmacodynamics (affinity and efficacy), its destruction of the cytochrome P_{450} enzymes (which delay the excretion of the nefopam and elevating its plasma concentration) and the damage proposed to the specific nefopam protein binding which all responsible for drug pharmacokinetics and disposition (10,11). Nefopam treatment at a subtle dose was found to cause stimulation of the central nervous system; leading to a stimulatory neurobehavioral effect in the chickens when examined at the open-field activity test with an enhanced stimulatory effect in the stressed chickens that may be referred to as receptor up-regulation because of OS status (24) or an increase in the release of the excitatory neurotransmitters (catecholamines) due to cellular damage that evokes a synergistic interaction to the nefopam mechanism of action. The serum ALP, ALT and AST concentrations in the normal (H_2O) group of chickens were found in normal ranges in the chicken as mentioned in the other study (25) and due to the ability of the H_2O_2 to cause cellular damage (like proteins), the liver function will also be affected which were found here through measuring the level of serum ALT, AST, and ALP concentrations in the stressed chickens and compare it to the normal one.

Conclusion

The sum of data findings indicated that H_2O_2-induced OS increased the analgesic activity of nefopam in the chickens; despite the changes occur on the neurobehaviour and liver function. The dose of nefopam should be reduced when preparing the therapeutic regimen in the stressed animals.

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Conflict of interest

The authors declare there is no conflict of interest.

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تأثير النيفوبام في الدجاج الطبيعي وعلاقته مع حالة الإجهاد التأكسدي المحدث ببيروكسيد الهيدروجين

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الخلاصة

هدف الدراسة صمم للكشف عن التأثير المسكن للألم لعقار النيفوبام في الدجاج الطبيعي (غير المجهد) ومعرفة التغير في هذا التأثير في حالة الإجهاد التأكسدي المحدث ببيروكسيد الهيدروجين في الدجاج بعمر ٧-١٤ يوم. زاد التأثير المسكن للنيفوبام بنسبة ٤٧% في الدجاج المجهد من خلال قياس الجرعة الفعالة الوسطية له إذ أن هذه الجرعة كانت ٩,١٠ ملغم/كغم، في العضل في الدجاج الطبيعي وقد أصبحت ٤,٨٠ ملغم/كغم، في العضل في الدجاج المجهد. لوحظ أن هناك زيادة معنوية في التأثير المسكن للنيفوبام بجرعة ١٨ ملغم/كغم، في العضل بنسبة ٨٨% في الدجاج المجهد مقارنة مع الدجاج الطبيعي من خلال قياس الألم باختبار التحفيز الكهربائي والفورمالديهايد ٠,٠٥ مل من ٠,١%. كما لوحظت تغييرات معنوية تحفيزية في السلوك العصبي للدجاج المجهد عند حقن النيفوبام بجرعة كامنة ١ ملغم/كغم، في العضل بالنسبة لوقت بدء الحركة، عدد المربعات المقطوعة ووقت الاستجابة لعدم الحركة الشدي. وعند إعطاء النيفوبام بجرعة ١٨ ملغم/كغم، في العضل، اكتشف أن هناك ضرر معنوي في الكبد من خلال قياس إنزيم الالكلاين فوسفاتيز، النين ترانساميناز واسبارتيت ترانساميناز في الدجاج المجهد ببيروكسيد الهيدروجين مقارنة مع تلك القيم في الدجاج الطبيعي وبنسبة ٢٨، ٦٥ و ٣٣% على التوالي. مجمل البيانات المتحصلة من هذه الدراسة تؤكد على أن الإجهاد التأكسدي المحدث ببيروكسيد الهيدروجين في الدجاج يعمل على زيادة التأثير المسكن للألم للنيفوبام بجانب التأثيرات الضارة في السلوك العصبي والضرر في وظيفة الكبد ويجب تقليل جرعة النيفوبام عند تحضير الجرع الدوائية في الحيوانات المجهدة.

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